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POSSIBLE PATHWAYS OF ARACHIDONIC ACID LIBERATION AS STUDIED WITH PURIFIED PHOSPHOLIPASES. H. Chap, B. Perret, G. Mauco, M. Plantavid, F. Laffont, M.F. Simon and L. Douste-Blazy.
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Two kinds of informations about arachidonic acid (AA) metabolism in platelet phospholipids (PL) have been obtained from the use of purified phospholipases :

1) Beside the determination of PL sidedness in the plasma membrane, non-lytic degradation by phospholipase A_2 + sphingomyelinase C showed that only 6 % of the total platelet AA is localized in the outer surface of the plasma membrane. This heterogeneous distribution is actually a consequence of PL asymmetry, since sphingomyelin and phosphatidylcholine, which predominate in membrane outer leaflet, contain only traces or relatively lower amounts, respectively, of AA than the internal lipids. It is further shown that incubating platelets with free AA specifically labels the large internal pool of AA, whereas the small external pool is renewed by a direct exchange of phosphatidylcholine with plasma lipoproteins. This offers a double-labelling method allowing to explore the exact role of each AA pool.

2) Platelet aggregation by *Clostridium welchii* phospholipase C produces the same metabolic changes (accumulation of phosphatidic and lysophosphatidic acids) as those induced by thrombin. These observations have led to describe the existence of a cytosolic phosphatidylinositol-specific phospholipase C and a membrane-bound diglyceride lipase. Both enzymes, coupled to diglyceride- (and monoglyceride-) kinase(s), could achieve AA release and (lyso) phosphatidic acid accumulation. Some properties of these enzymes (subcellular localization, calcium and pH dependence, positional specificity) will be presented.

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PHOSPHOLIPID TOPOGRAPHY COAGULATION

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Phospholipids play a role in thrombin generation because the constitute the support on which both the factor X activating enzyme ("tenase") and the prothrombin activating enzyme (prothrombinase) are built.
In model systems with pure synthetic phospholipids it can be shown that the binding of the coagulation factors to a phospholipid surface is a function of both phospholipid charge and membrane fluidity. A relatively high molefraction of phosphatidyl serine (PS) is a prerequisite for optimal binding of the vitamin K dependant factors. Precise measurements on the binding constant and the number of binding sites of factor X show that at molefractions of PS between 0.01 and 0.25 aproximately one PS binds per Gla-residue. Above 0.25% of PS vesicle agregation occurs.
The normal intact circulating platelet shows a molefraction of PS of $\sim$ 0.03 in the outer leaf let of its plasma membrane and therefore hardly if at all stimulates thrombinformation.
Upon activation by thrombin (2nM) together with collagen, (10 μg/ml) the outer leaflet aquires increasing amounts of PS without platelet disruption taking place.
This suggests a flip-flop movement of phospholipids across the membrane as part of the activation reaction of platelets. It can be shown that optimal "tenase" supporting phospholipid compositions appear before prothrombine supporting ones. The activation by thrombin and collagen is only partly inhibited by prostacyclin even at high concentrations.